Understanding sperm DNA fragmentation

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We greatly value the commentary by Dr. Gosalvez on the "Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios" by Agarwal *et al.* (1). The author initiated his well-written note with an overview of the structural and physiologic significance of sperm DNA on male reproduction addressing its association with varicocele, recurrent spontaneous abortion and unexplained infertility, and its implications on embryonic development and fecundity both naturally and after assisted reproductive techniques (ART). Throughout his discussion, Dr. Gosalvez rightly highlighted the importance of understanding the etiology, root causes and types of sperm DNA fragmentation (SDF) as this would ultimately influence interpretation of SDF test result and our understanding of the novel treatment methods utilized in such circumstances. We want to seize this opportunity to elaborate on this particular issue.

Mammalian sperm DNA is unique in such a way that it is highly organized, condensed and compacted. In contrast to the DNA structure of somatic cells which is wrapped around an octamer of histones, packaged into nucleosomes and coiled into a solenoid thereby increasing the chromatin volume (2), sperm DNA is rather hindered by the confined sperm nuclear space and hence undergoes necessary modifications to its packaging process. During spermiogenesis histones are lost and replaced with transition proteins and subsequently with protamines (3). Cystein residues of the much smaller protamines further undergo intra- and inter-molecular disulfide cross linking resulting in a highly condensed chromatin arranged in a toroid (4). This complex packaging and compaction affords necessary protection to sperm chromatin during its transport from the male to female reproductive tracts and ensures delivery of paternal genetic material to the developing embryo.

Although human sperm DNA undergoes the same structural modifications as described above, it is less compact than the sperm DNA of other mammals. A certain amount of histones is retained in human sperm chromatin making it less compact and subject to injury (5). In fact, a higher histone to protamine ratio has been detected in men complaining of infertility (6,7). Furthermore, two types of protamines, P1 and P2, exist with the latter containing fewer cysteine groups and hence less disulfide crosslinks thereby theoretically making the sperm DNA more susceptible to damage (8). While assessing sperm DNA integrity in infertile men with varicocele, Ni *et al.* reported a significant reduction in protamine 1/2 mRNA ratio in patients with clinical grade 3 varicocele (9).

Different types of SDF such as single- or double-stranded DNA breaks, DNA nicks, nuclear protein defects, and alteration of chromatin configuration can occur secondary to a wide variety of etiologic factors. Disease states such as varicocele, infections and inflammations of the genital tract, cancer, genetic mutations, chromosomal abnormalities and environmental and habitual exposures have all been identified resulting in DNA damage to the sperm either during spermiation or during its transit through the male reproductive tract (10-12). Intra-testicular damage is believed to occur secondary to abortive apoptosis or to alteration in sperm maturation. Under normal physiologic circumstances, almost half of germ cells entering meiosis I of spermatogenesis are exposed to markers of the Fas type and hence undergo abortive apoptosis and are expelled by Sertoli cells (13). However, under pathologic conditions

the defective germ cells may not be efficiently eliminated with a variable percentage of them entering spermiogenesis and appearing in the ejaculate (14). Evidence suggest that these defective gem cells may in fact have a normal morphologic appearance after spermiogenesis despite their poor genomic quality, a fact that further complicates the situation and highlights the importance of SDF testing (15). Alteration in sperm maturation is another theory that is thought to result in intra-testicular damage to sperm DNA. During spermiogenesis, DNA strand nicks/breaks occur to facilitate the process of protamination which would later result in highly compact sperm DNA as previously mentioned. Defects in the enzymatic repair process of these DNA breaks are believed to be responsible for alteration in sperm maturation resulting in dramatic consequences to the genomic integrity of the sperm cell (16).

Recent evidence indicates that the intra-testicular mechanisms of DNA damage are not solely responsible for the appearance of SDF in the ejaculate. This hypothesis was based on the findings of few reports which revealed a higher incidence of SDF in ejaculated and epididymal sperm than in testicular sperm (17-19). Oxidative stress induced DNA damage has been considered as the principle factor in this regard. Excessive quantities of reactive oxygen species (ROS) could be produced by immature spermatozoa, inflammatory cells and epididymal epithelium resulting in DNA damage of mature sperm cells. This is consistent with the results of *in vitro* studies, demonstrating ROS-induced SDF after exposure of mature sperm cells to ROS (20), and of *in vivo* studies conveying the presence of a significant positive correlation between SDF and markers of oxidative stress (21,22). These facts in addition to the compelling evidence provided by recent meta-analyses (23-25) indicating an increased risk of pregnancy loss after intracytoplasmic sperm injection (ICSI) in patients with high SDF, triggered researchers to explore the utility of testicular sperm instead of ejaculated sperm in patients with high SDF. Esteves *et al.* (19) compared the clinical outcome of ICSI performed with ejaculated sperm or testicular sperm in 147 men with high SDF. The percentage of SDF, measured with a variant of the Halosperm test, was significantly lower in testicular sperm in comparison to ejaculated sperm of the group undergoing testicular sperm retrieval (P<0.001). More importantly, the authors revealed a statistically significant lower miscarriage rate (P=0.012) and higher live birth rate (P=0.007) in the testicular sperm group in comparison to the ejaculated sperm group. In another study, Arafa *et al.* (26) compared the clinical outcome of a consecutive testicular sperm ICSI cycle performed on 36 men with high SDF to the clinical outcome of a prior ejaculated sperm ICSI cycle. These authors also demonstrated significantly higher pregnancy rates (38.9% *vs.* 13.5%, P<0.001) and live birth rates (47.2% *vs.* 8.3%, P<0.001) with the testicular sperm in comparison to the ejaculated sperm ICSI cycle.

Dr. Gosalvez has indicated in his commentary that SDF may in fact play an important role in embryo quality and early embryonic loss stressing on the importance of future research in this area. We agree with his view on this as demonstrated by recent publications. Simon *et al.* (27) evaluated embryo quality after ICSI performed with semen samples from 215 men with low or high SDF. Using single cell gel electrophoresis to detect the degree of DNA damage, men with lower levels of SDF had a significantly higher percentage of good quality embryos and lower percentage of poor quality embryos (P=0.05) in comparison to men with high SDF. In another study, Wdowiak *et al.* (28) investigated the dynamics of SDF and its implication on embryo development and pregnancy rate. The authors utilized sperm chromatin dispersion test on semen samples from 148 couples undergoing ICSI measuring SDF level at various time periods (initially and after 3, 6 and 12 h of incubation). The SDF level and the intensity of fragmentation was correlated with embryo growth and pregnancy outcome. The authors observed that the early stages of embryo development were dependent on the initial SDF level, and that further progression of embryo development into pregnancy depends on the amplification of SDF after 12 h of incubation. They detected a 5.9% lower likelihood of pregnancy for every 1 unit increase in SDF observed after 12 h of incubation.

Finally, we believe that the proper utility of SDF testing could provide valuable information that may change the clinical outcome of the couple seeking fertility. Perhaps there is no better way to conclude this reply than to quote Dr. Gosalvez's ending statement "it is the information about the quality of DNA that is going to be most relevant for subsequent syngamy and embryogenesis".

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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